65. The method of claim 62, wherein said polynucleotide is introduced into said cell using a nonviral vector.

66. The method of claim 65, wherein said nonviral vector is introduced into said cell as naked DNA or using liposome-mediated transfection.

REMARKS

Claims 1-46 are pending in this application. Applicants have cancelled claims 1-17 and added new claims 47-66. No new matter has been introduced with the foregoing amendments. Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made." Reconsideration is respectfully requested.

I. The Invention

The present invention is directed to a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal. The invention is also directed to a method of inhibiting the generation of active thrombin on the surface of a cell within a mammal by introducing into said cell a polynucleotide encoding an ER resident chaperone protein.

II. Rejection under 35 U.S.C. § 112, second paragraph

Original claims 11-15 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants have replaced claims 11-15 with new claims 55-59 and 62-66. Claims 55 and 62 recite "introducing into said cell a polynucleotide operably linked to a promoter, wherein said polynucleotide encodes said ER resident chaperone protein", thereby making clear that

the promoter is operably linked to the polynucleotide and not the protein. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

III. Rejection under 35 U.S.C. § 112, first paragraph, written description

Original claims 1-5 and 8-17 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. To the extent that this rejection applies to new claims 47-66, Applicants respectfully traverse because an "ER resident chaperone protein" is a genus that is represented by the specific ER resident chaperone proteins recited in the specification.

Independent claims 47 and 62 each recite a method of inhibiting the generation of active thrombin on the surface of a cell that requires the step of producing an ER resident chaperone protein in that cell. As can be seen by the attached definition for "chaperones" from *The Dictionary of Cell and Molecular Biology*, this word is a term of art that defines a family of proteins based on the functional ability to assist in the proper folding of other proteins. Applicants' claimed methods use those chaperone proteins that are resident to the endoplasmic reticulum.

The Examiner states on page 5 of paper 9 that Applicants describe the function common to all ER resident chaperone proteins. As the Examiner points out, Applicants have defined "ER resident chaperone protein" on page 10, lines 11-13 of the specification to refer to any protein, present in, or associated with, the ER, that acts to facilitate the folding, assembly, or translocation of proteins.

Applicants respectfully submit that the Examiner's citation of *The Regents of the University of California v. Eli Lilly and Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997) and *Fiers v. Sugano*, 25 USPQ2d 1601 is improper and out of context in the present rejection because the claims at issue in the cited cases were directed to specific nucleic acid sequence *compositions* based on methods of obtaining them. The instant claims are

distinguishable, because Applicants claim *methods* that use ER resident chaperone proteins having sequences that are in the public domain, wherein such ER resident chaperone proteins are defined based on a function recognized by and known to those in the art.

Applicants teach on page 16, lines 3-15 of the specification, that the claimed methods can use any known ER resident chaperone protein. Applicants provide on pages 46 and 47 of the specification the nucleic acid and amino acid sequences of the ER resident chaperone protein GRP78/BiP, which were in the public domain (GenBank Accession number AJ271729). The specification additionally provides on page 16, lines 6-13 references to the sequences of other ER resident chaperone proteins that can be used in the present invention. For example, GRP94 (GenBank Accession No. M26596), calnexin (GenBank Accession No. M94859), calreticulin (GenBank Accession No. NM_004343), and reticulocalbin. A sequence of human calnexin (IP90) (GenBank Accession number L10284) was also in the public domain. Other ER resident chaperone proteins, that by a definition understood to those in the art, function to facilitate the folding, assembly, or translocation of other proteins, can be used in the practice of the claimed methods once they are known.

The Examiner particularly objects that the definition of an "ER resident chaperone protein" on page 10 of the specification encompasses variants and derivatives thereof, stating that this definition comprises hundreds of millions of different possibilities. Applicants respectfully submit that the Examiner is incorrect. It is indicated on page 13, line 9, through page 15, line 8 of the specification that the nucleic acid and amino acid sequences of known ER resident chaperone proteins for use in the claimed methods also encompass conservatively modified variants thereof. The specification states on page 13, lines 10-11, and on page 14, lines 8-23 that conservatively modified variants of a nucleic acid sequence include degenerate codon substitutions, complementary sequences, and those nucleic acid sequences which encode identical or essentially identical sequences. Conservatively modified variants of amino

acid sequences are defined on page 14, line 24 through page 15, line 8 as deletions or additions to a nucleic acid or polypeptide sequence which alters, adds, deletes or conservatively substitutes a single amino acid or a small percentage of amino acids in the encoded sequence. Amino acid analogues that have the same basic chemical structure as a naturally occurring amino acid and amino acid mimetics that function in a manner similar to the naturally occurring amino acids are also encompassed within amino acid variants or derivatives. Contrary to the Examiner's assertions, Applicant's definitions of variants and derivatives provided on page 13, line 9 through page 15, line 8 would indicate closely related nucleic acid and amino acid structures that function similarly to the publicly disclosed sequences of ER resident chaperone proteins for use in the claimed methods.

Therefore, in view of the foregoing, Applicants respectfully assert that the specification sufficiently conveys to those of skill in the art that at the time of filing that Applicants were in possession of a method of inhibiting the generation of active thrombin on the surface of a cell by producing an ER resident chaperone protein in said cell. Applicants' invention resides in their novel methods that use known sequences encoding an ER resident chaperone protein, and variants and derivatives thereof, which function according to the definition known to those in the art in facilitating the folding, assembly or translocation of proteins. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

IV. Rejection under 35 U.S.C. § 112, first paragraph, enablement

Original claims 1-15 stand rejected under 35 U.S.C. § 102(b) as allegedly including subject matter that was not described in the specification in such a way as to enable one of skill in the art to make and/or use the invention. In response, Applicants respectfully traverse the rejection.

Independent claim 62 specifically recites and independent claim 47 encompasses a method of inhibiting the generation of active thrombin on the surface of a

cell by introducing to that cell a polynucleotide encoding an ER resident chaperone protein. The Examiner objects to methods that recite introducing an exogenous ER resident chaperone into a cell *in vivo* (see page 6 of paper 9), and cites *In re Wands*, 8 USPQ2d 1400 to support this objection. Applicants respectfully submit that the *Wands* factors support that Applicants have enabled those skilled in the art to practice the claimed methods.

The nature of the invention

The claims are directed to methods of inhibiting the generation of active thrombin on the surface of a cell by producing an ER resident chaperone protein in that cell, where producing can intend introducing into the cell a polynucleotide that encodes an ER resident chaperone protein.

The breadth of the claims

The claims encompass using a polynucleotide encoding an ER resident chaperone protein, or any conservatively modified variant or derivative thereof that retains structural similarity and the understood function of an ER resident chaperone protein. The specification teaches that inhibiting the generation of active thrombin on the surface of a cell is applicable to any of a number of thrombotic diseases (*see* page 9, lines 17-33), including coagulation disorders, cardiovascular diseases, sepsis, cancer and radiation-related thrombotic events.

Working examples and guidance in the specification

The examples provided on pages 37-45 demonstrate inhibition of thrombin generation on the surface of cultured human bladder carcinoma cells by introducing a nucleic acid sequence encoding GRP78/BiP by liposomal transfection. Additionally, Applicants provide detailed guidance on page 18, line 26 through page 21, line 3 for available methods of introducing polynucleotides into cells *in vivo*. Applicants cite here numerous references that provide protocols for successful viral and non-viral delivery of a nucleic acid encoding an ER resident chaperone protein to cells *in vivo*.

The unpredictability of the art and the state of the prior art

Viral delivery methods are sufficiently predictable and successful that Applicants cite 20 references which provide specific parameters for using a particular viral vector to introduce an ER resident protein into a cell. Non-viral methods are sufficiently predictable and successful that Applicants cite 19 references which provide specific protocols for non-viral delivery of DNA to a cell. The examiner quotes from Crystal (1995) *Science* 270:404 and Walther and Stein (2000) *Drugs* 60:249 to support the position that the state of the art of gene therapy is plagued by unpredictability. However, Crystal also states in the abstract of the *Science* article that "[e]nough information has been gained from clinical trials to allow the conclusion that human gene transfer is feasible, [and] can evoke biologic responses that are relevant to human disease." Even Walther and Stein state in their *Drugs* article that "many achievements have been made in vector safety, the retargeting of virus vectors and improving the expression properties by refining the vector design and virus production." Applicants respectfully assert that the 39 cited references provide ample evidence of success using viral and non-viral modes of delivery, and their disclosures provide protocols that enable predictable application of viral delivery systems for different nucleic acid sequences of interest.

Quantity of experimentation

Because the numerous cited publications provide detailed guidance and specific parameters for viral and non-viral delivery of a nucleic acid to a cell in a mammal, the quantity of experimentation is minimized. Routine optimization with a different nucleic acid may be time consuming, but this does not constitute undue experimentation. The cited publications support the position that viral and non-viral *in vivo* delivery systems for nucleic acids are known in the art and have been used successfully.

Level of skill in the art

The level of skill in the art is high.

Summary

Because numerous detailed protocols for viral and non-viral delivery of a nucleic acid to a cell are readily available in the public domain and references to such protocols are provided in the specification, introducing a nucleic acid sequence encoding an ER resident chaperone protein to a cell would not require undue experimentation. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

V. Rejection under 35 U.S.C. § 102(b) in view of Nakai, *et al*

Original claims 1, 4-8, 16 and 17 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Nakai, *et al*, *Cell Structure and Function* (1995) 20:33. To the extent that it applies to new claims 47-61, Applicants respectfully traverse this rejection because Nakai, *et al.* do not teach or suggest a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal. Furthermore, Nakai, *et al.* do not teach or suggest a method of inhibiting the generation of active thrombin on the surface of a cell within a mammal by introducing into said cell a polynucleotide encoding a ER resident chaperone protein as recited in new claims 62-66.

For a rejection under 35 U.S.C. 102(b) to be proper, each and every element as set for the in the claim must be found, either expressly or inherently described, in a single prior art reference. M.P.E.P. § 2131.

Nakai, *et al.* disclose that administration of interleukin-6 to cultured mouse myeloid leukemia cells induces differentiation to a macrophage-like phenotype and correlates with increased synthesis of BiP/GRP78 and GRP94. Nakai, *et al.* do not teach or suggest or mention administration to any cell within an atherosclerotic plaque within a mammal, as recited in claim 47, or administration to any cell within a mammal by introducing a nucleic acid encoding an ER resident chaperone protein, as recited in claims 55 and 62. Nakai, *et al.* disclose a basic scientific *ex vivo* study of induction of differentiation in cultured murine myeloid leukemia cells, but do not teach or suggest any

method of inhibiting the generation of active thrombin in a cell within a mammal, which are required elements of independent claims 47 and 62.

Because Nakai, *et al* fail to teach or suggest each and every element of independent claims 47 and 62, and therefore each and every element of dependent claims 48-61 and 63-66, it is not possible for this cited reference to anticipate the claimed methods. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

VI. Rejection under 35 U.S.C. § 102(b) in view of Cheng, *et al*

Original claims 1-3, 7-9 and 16 stand rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Cheng, *et al*, *Int. J. Exper. Path.*, *79*:125 (1995). To the extent that it applies to new claims 47-61, Applicants respectfully traverse this rejection because Cheng, *et al.* do not teach or suggest a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal. Furthermore, Cheng, *et al.* do not teach or suggest a method of inhibiting the generation of active thrombin on the surface of a cell within a mammal by introducing into said cell a polynucleotide encoding a ER resident chaperone protein as recited in new claims 62-66.

Cheng, *et al* disclose administering gentamycin to rats and use immunohistochemistry to detect correlative increases in expression of heat shock proteins HSP47 and HSP72/73 in tubular epithelial cells and myofibroblasts (α-smooth muscle cells) of kidney tissue during fibrosis. Cheng, *et al* do not teach or suggest or mention administration to any cell within an atherosclerotic plaque within a mammal, as recited in claim 47, or administration to any cell within a mammal by introducing a nucleic acid encoding an ER resident chaperone protein, as recited in claims 55 and 62. Cheng, *et al.* is a basic research study that observes the expression pattern of HSP47 in gentamycin-treated kidney tissue, but fails to teach each and every element of independent claims 47 and 62.

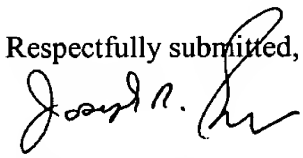
Accordingly, because Cheng, *et al.* cannot possibly anticipate independent claims 47 and 62, and therefore dependent claims 48-61 and 63-66, the Examiner is respectfully requested to withdraw this rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Paragraphs beginning at page 5, line 26 through page 6, line 16 have been amended as follows:

Figure 3. Overexpression of GRP78/BiP suppresses total (A) and free (B) thrombin generation on T24/83 cell surfaces. Normal pooled human plasma was used to measure total and free thrombin generated on the surface of T24/83 cells. Wild type (▲), vector transfected ([O] ■), or GRP78/BiP overexpressing cells ([□] ●). Data represent mean ∀ SEM (standard error of the mean, *i.e.*, the standard deviation divided by the square root of sample size) of triplicate measurements from four separate experiments. GRP78/BiP overexpressing cells generated significantly less thrombin compared with wild-type or vector-transfected cells ($p<0.001$).

Figure 4. Overexpression of GRP78/BiP decreases prothrombin consumption on T24/83 cell surfaces. Normal pooled human plasma was used to measure prothrombin consumption on the surface of T24/83 cells. Wild type (▲), vector transfected ([O] ■), or GRP78/BiP overexpressing cells ([□] ●). Data represent mean ∀ SEM of triplicate measurements from four separate experiments. GRP78/BiP overexpressing cells consumed significantly less prothrombin after 4 min, compared with wild-type or vector-transfected cells ($p<0.001$).

Figure 5. Effect of GRP78/BiP overexpression on free thrombin generation in normal (A) or factor VII-deficient (B) plasma containing APTT reagent. Normal or factor VII-deficient human plasma, in the presence of APTT reagent, was used to measure free thrombin generation on the surface of T24/83 cells. Wild type ([□] ▲), vector transfected ([O] ■), or GRP78/BiP overexpressing cells ([□] ●). Data representing mean ∀ SEM of triplicate measurements from four separate experiments. In the presence of normal (A), but not factor VII-deficient plasma (B), peak free thrombin

generation was significantly decreased in the GRP78/BiP overexpressing cells, compared to wild-type or vector-transfected cells ($p<0.001$).

In the claims:

Claims 1-17 have been cancelled.

Claims 47-66 have been added as follows:

47. A method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal, the method comprising producing an ER resident chaperone protein in said cell within an atherosclerotic plaque within said mammal.

48. The method of claim 47, wherein said cell is an endothelial cell.

49. The method of claim 47, wherein said cell is a smooth muscle cell.

50. The method of claim 47, wherein said cell is a macrophage.

51. The method of claim 47, wherein said cell is a monocyte.

52. The method of claim 47, wherein said ER resident chaperone protein is GRP78/BiP.

53. The method of claim 47, wherein said ER resident chaperone protein is selected from the group consisting of GRP94, GRP72, Calreticulin, Calnexin, Protein disulfide isomerase, cis/trans-Prolyl isomerase, and HSP47.

54. The method of claim 47, wherein the production of said ER resident chaperone protein within said cell results in a decrease in the level of tissue factor procoagulant activity on the surface of said cell.

55. The method of claim 47, wherein a polynucleotide operably linked to a promoter is introduced into said cell, wherein said polynucleotide encodes said ER resident chaperone protein, whereby said ER resident chaperone protein is produced.

56. The method of claim 55, wherein said polynucleotide is introduced into said cell using a viral vector.

57. The method of claim 56, wherein said viral vector is an adenoviral vector.

58. The method of claim 55, wherein said polynucleotide is introduced into said cell using a nonviral vector.

59. The method of claim 58, wherein said nonviral vector is introduced into said cell as naked DNA or using liposome-mediated transfection.

60. The method of claim 47, wherein said ER resident chaperone protein is produced by administering to said cell a compound that induces the expression or activation of an endogenous ER resident chaperone protein.

61. The method of claim 60, wherein said compound is a cytokine.

62. A method of inhibiting the generation of active thrombin on the surface of a cell within a mammal, the method comprising producing an ER resident

chaperone protein in said cell within said mammal by introducing into said cell a polynucleotide operably linked to a promoter, wherein said polynucleotide encodes said ER resident chaperone protein, whereby said ER resident chaperone protein is produced.

63. The method of claim 62, wherein said polynucleotide is introduced into said cell using a viral vector.

64. The method of claim 63, wherein said viral vector is an adenoviral vector.

65. The method of claim 62, wherein said polynucleotide is introduced into said cell using a nonviral vector.

66. The method of claim 65, wherein said nonviral vector is introduced into said cell as naked DNA or using liposome-mediated transfection.

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chaperones

Cytoplasmic proteins of both prokaryotes and eukaryotes (and organelles such as mitochondria) that bind to nascent or unfolded polypeptides and ensure correct folding or transport. Chaperone proteins do not covalently bind to their targets and do not form part of the finished product. Heat-shock proteins are an important subset of chaperones. Three major families are recognized, the chaperonins (groEL and hsp60), the hsp70 family and the hsp90 family. Outside these major families are other proteins with similar functions including nucleoplasmin, secB, and T-cell receptor-associated protein.

Author: John Lackie

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